Fetal Diagnosis and Therapy

Fetal Diagn Ther 2006;21:458–465
DOI: 10.1159/000093889

Received: August 20, 2005
Accepted: November 12, 2005

Fetal Myocardial Thickening Measured by Ultrasonic-Based Technique Called 'Phased-Tracking Method'

Shutaro Kunii[a] Junichi Sugawara[a] Yoshitaka Kimura[a] Noriaki Imai[a] Hiroshi Chisaka[a] Hideyuki Hasegawa[c] Yoshiro Koiwa[b] Hiroshi Kanai[c] Kunihiro Okamura[a]

Departments of [a]Obstetrics and Gynecology, [b]Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, [c]Department of Electronic Engineering, Graduate School of Engineering, Tohoku University, Sendai, Japan

Key Words

Fetus · Ultrasonics · Myocardial contraction · Heart failure · Imaging

Abstract

Objective: To evaluate fetal myocardial movement by using newly developed ultrasonic technique. ***Methods:*** We analyzed 50 normal fetuses between 25 and 41 weeks' gestation for changes in thickness of fetal myocardium using the phased-tracking method, a technique with high vertical distance resolution and the potential to evaluate fine ventricular wall movements. We analyzed differences in the rate of change in ventricular wall thickness and in changes in the inner and outer wall layers with advancing gestation. We also analyzed myocardial thickening period and evaluated the ratio of increasing thickness period to stroke interval. ***Results:*** Mean thickness changing rate was significantly higher in the right (1.18 ± 0.34 m/s/m) than in the left ventricular wall (0.86 ± 0.31 m/s/m) ($p < 0.001$). Mean ratio of increasing thickness period to stroke interval was significantly higher in the right (0.57 ± 0.064) than in the left ventricle (0.46 ± 0.075) ($p < 0.001$), indicating that myocardial contraction in the fetal right ventricle predominates. The thickness-changing rate of the bilateral ventricular walls was positively and linearly correlated with gestational age. The myocardial-wall thickness-changing rate was higher in the outer layer than in the inner layer in late gestation. ***Conclusions:*** We conclude that measurement of the thickness-changing rate of fetal ventricular walls using the phased-tracking method might be useful for evaluation of fetal cardiac function.

Introduction

Ultrasonic evaluation of fetal cardiac function is widely used, simple, and non-invasive. The major methods in the clinical setting are M-mode echocardiography and an indirect evaluation using the hemodynamic Doppler effect. Because the widths of areas for measurement are considerably smaller in fetuses than in adults, measurement errors and interexaminer differences are not negligible [1–3]. Cardiac function originates from myocardial contractility, and reduction of myocardial movement by insults such as hypoxia is thought to precede reduction of output. However, there have been no methods to date that allow observation of precise myocardial movements

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–3837/06/0215–0458$23.50/0

Accessible online at:
www.karger.com/fdt

Junichi Sugawara, MD
Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology
Tohoku University Graduate School of Medicine, 1-1, Seiryomachi, Aobaku
Sendai 980-8574 (Japan)
Tel. +81 22 717 7254, Fax +81 22 717 7258, E-Mail sugawara@mail.tains.tohoku.ac.jp

in fetuses. The methods for evaluation of cardiac function in fetuses are thus quite limited compared to those available for adults. Development of a method for direct evaluation of myocardial movement in fetuses will be very useful.

Recently, there have been studies on tissue Doppler echocardiography using Doppler signals for evaluation of myocardial movement in fetuses [4–7]. However, changes in myocardial thickness and cardiac movement by pulsation are measured without distinction by tissue Doppler echocardiography and changes in myocardial thickness alone are not recorded, making the method of limited value for evaluation of myocardial dynamics.

Kanai et al. [8] reported a tracking method by which measurement points of reflected ultrasonic pulsatile waves fixed in myocardium were accurately examined. They also developed a modified phased-tracking method with which changes in myocardial thickness are continuously measured by integrating the difference in velocity between two points along an ultrasonic beam. The time and spatial resolutions of the phased-tracking method are very high, and changes in the thickness of cardiac muscle can be measured at a level of several micrometers [9]. Koiwa et al. [10] clinically examined the toxicity of doxorubicin hydrochloride in adult myocardium with use of the phased-tracking method, and reported that the detection of myocardial microdisorders by this method may be more sensitive than that by other ultrasonic measurement methods. Impairment of cardiac function may also be observed in fetuses as changes in myocardial contraction.

In the present study, we evaluated whether changes in the thickness of fetal myocardium could be measured by the phased-tracking method, and we also examined differences in the thickness-changing rate between the bilateral ventricular walls and between the inner and outer layers of the ventricular wall based on weeks of gestation. Furthermore, we analyzed the myocardial thickening period and evaluated the ratio of myocardial contraction time (t) to stroke interval (T) ratio of increasing thickness period to stroke interval (RITS).

Materials and Methods

Subjects

Between January 2003 and December 2003 we examined 50 normal fetuses between 25 and 41 weeks of gestation (mean, 33.5 ± 3.9 weeks). In all fetuses, gestational age was calculated from the first day of the last menstrual period and confirmed by ultrasound examination between 9 and 11 weeks of gestation. Inclusion criteria for normal fetuses were singleton pregnancy, absence of fetal growth restriction, absence of fetal structural malformations on ultrasound and later at birth, and absence of maternal disease affecting fetal growth. The mothers' age range was 19–37 years (mean, 28.5 ± 5.2 years). The study design was approved by the institutional review board, and all women gave informed consent before participating in the study.

Measurement of Myocardial Layer Thickening

Using ultrasonic diagnostic equipment (Toshiba, SSA340A, Tokyo, Japan), the direction of the ultrasonic beam passing through the measurement points was selected in the four-chamber view, B-mode cross-sectional image so as to be almost perpendicular to the right ventricular (RV) wall and left ventricular (LV) wall during measurement. Just after selection, the direction of the ultrasonic beam was fixed and the quadrature-demodulated signal of the RF signal was A/D converted during several heartbeats. The principles of the phased-tracking method, including theoretical and in vivo evaluations for myocardial layer thickening rate, have been detailed previously [8, 9]. In brief, radiofrequency pulses with an angular frequency of $\omega_0 = 2\pi f_0$ are transmitted at time intervals of ΔT from an ultrasonic transducer on the maternal abdominal wall. The phase difference, $\Delta\theta(x;t)$, between the phase $\theta(x;t)$ of the quadrature-demodulated signal of the received signal, $y(x;t)$, and the phase $\theta(x;t + \Delta T)$ of the quadrature-demodulated signal of the subsequently received signal, $y(x;t + \Delta T)$, is given by

$$\Delta\theta(x;t) = \theta(x;t + \Delta T) - \theta(x;t) = -2\omega_0/c_0 \cdot \Delta x(t) \quad (1)$$

where $\Delta x(t) = x(t + \Delta T) - x(t)$ is the movement of the object during the period ΔT around time t, and c_0 is the acoustic velocity in the human body. In our examination, the period ΔT was set at 250 μs. Because the maximum value of the acceleration in the interventricular septum (IVS) is about 4 m/s^2, the velocity of the adult IVS changes at most by 0.001 m/s, which is 1/400 of the higher limit of the measurable velocity (0.4 m/s) for the short period ΔT of 250 μs. Thus, the velocity of the IVS is assumed to be constant during the pulse-repetition interval ΔT. By dividing the movement Δx by the period ΔT, the average velocity $v(t + \Delta T/2)$ of the object during the period ΔT is given by

$$v(t + \Delta T/2) - \Delta x(t)/\Delta T = c_0/2\omega_0 \cdot \Delta\theta(x;t)/\Delta T \quad (2)$$

The phase difference $\Delta\theta(x;t)$ is accurately determined by the constraint least squares approach based on the complex crosscorrelation between $y(x;t)$ and $y(x;t + \Delta T)$ under the condition that signal waveforms change only in phase values during the period ΔT. It is impossible to accurately determine the true lag value from the complex correlation function, which is derived from the standard normalized mean squared difference between the quadrature-modulated signals of the successively received signals [11]. However, introduction of the constraint is effective for determination of the lag between these complex signals. By multiplying the resultant velocity $(t + \Delta T/2)$ by the period ΔT, the next depth $x(t + \Delta T)$ of the object is estimated by

$$x(t + \Delta T) = x(t) + v(t + \Delta T/2) \times \Delta T \quad (3)$$

Using the resultant signal $x(t + \Delta T)$, the displacement of the object (the position of the region of interest) in the heart wall is successfully tracked, and the velocity signal $v|Ax(t)$ on the large motion due to the heartbeat is accurately measured.

In the current study, a high-speed A/D converter with large-scale memory was employed to analyze the complex signal resulting from the quadrature modulation of the signal received by a sector-type ultrasonic transducer connected to standard ultrasonic diagnostic equipment. We used an ultrasonic frequency of 3.75 MHz, with a pulse repetition interval of 222 μs. The resultant real and imaginary signals of the demodulated Doppler signal were simultaneously A/D converted with a two-channel 12-bit A/D converter at a sampling rate of 1 MHz. The initial positions of the multiple sampling points across the wall were manually set along the ultrasonic beam. The lower limit of resolution of the change in layer thickness was 0.5 μm and the higher limit of measurable velocity was about 0.4 m/s, which was determined by aliasing with the conventional Doppler system [8]. Measurement was performed in resting fetuses, taking effects of fetal motions such as respiration-like movement on the cardiovascular system into consideration, and the mean measurements in three cardiac cycles were analyzed. Measurement was performed at the site perpendicular to the IVS at the height of the tricuspid valve tip.

To visualize changes in thickness, the ventricular wall was divided into layers 0.75 mm thick, and thickness changing rate was expressed with colors. Blue indicated an increasing thickness of myocardium, whereas red and yellow indicated thinning. Light blue and yellow indicated more rapid myocardial changes. We examined whether homogeneous changes in fetal myocardial thickness corresponded with cardiac cycle stages and numerically evaluated observed myocardial thickening changes. The mean time (t) between starts of myocardial thickening and thinning was determined using the thickness-changing rate measured at intervals of 222 μs (fig. 1). Analysis of the RV and LV walls was separately performed, covering the entire ventricular wall (width, about 4.5 mm). The RITS of the RV and LV walls were compared (fig. 1). Furthermore, the relationship between gestational age and RITS was examined. The bilateral ventricular walls were divided into inner and outer layers at the center of the ventricular wall (width, about 2.25 mm), and their thickness-changing rate was separately determined to examine the relationship between thickness-changing rate and gestational age.

Statistics

All data are expressed as mean value ± standard deviation (SD). The Mann-Whitney test was used for two-way comparison of data. Values were considered significantly different at $p < 0.05$. Correlations between age in gestational weeks and measurement results were analyzed with use of linear regression analysis.

Results

Figure 2 indicates an example of the phased tracking-method as performed on a normal fetus at 30 weeks gestation. Homogeneous changes in myocardial thickness corresponding to cardiac cycle stages were observed in all fetuses.

The mean thickness changing rate in the 50 fetuses was 1.18 ± 0.34 m/s/m (mean ± SD) in the RV wall and 0.86 ± 0.31 m/s/m in the LV wall; the higher contraction rate of the RV wall was significant compared with that of the LV wall ($p = 0.0001$, fig. 3). Correlations between thickness changing rate of the bilateral ventricular walls and gestational age were positive and linear (RV wall, $y = 0.027x + 0.316$, $R^2 = 0.112$, $p = 0.018$; LV wall, $y = 0.024x + 0.094$, $R^2 = 0.102$, $p = 0.023$) (figs. 4, 5). The mean RITS was significantly higher in the RV (0.57 ± 0.064) than in the LV (0.46 ± 0.075) ($p < 0.0001$, fig. 6), showing that myocardial thickening was more rapid and longer in the RV wall than in the LV wall. There were no correlations between RITS and gestational age (RV, $p = 0.17$; LV, $p = 0.11$) (data not shown).

Figures 7 and 8 show the relationship between ratio of thickness-changing rate of the outer layer to that of the inner layer for the RV and LV walls and gestational age, respectively. The ratio was significantly correlated with gestational age in weeks (RV wall, $y = 0.11x - 2.65$, $R^2 = 0.403$, $p < 0.0001$, fig. 7; LV wall, $y = 0.146x - 3.534$, $R^2 = 0.323$, $p < 0.0001$, fig. 8).

Discussion

In this study, we measured microchanges in the thickness of local myocardium in normal human fetuses by the phased tracking method and found that thickening changes were more rapid and longer in the RV wall than in the LV wall. Furthermore, the thickness changing rate of the outer layer of the myocardium increased as gestation advanced.

Since M-mode echocardiography was applied to fetuses in 1970 [12], direct measurement and evaluation of changes in fetal myocardial thickness using ultrasound have been attempted. There have been a number of studies on the increasing rate of wall thickness ([wall thickness in systole – wall thickness in diastole]/wall thickness in diastole n 100) determined by measuring myocardial thickness at the end of diastole and systole by M-mode echocardiography. Sutton et al. [13] reported that there were no differences in the increasing rate of wall thickness of the RV wall and IVS between 20 and 40 weeks of gestation, but that the rate for the LV wall was significantly lower at 20 weeks than at 40 weeks of gestation; they suggested that the dominance of the LV is reduced with fetal maturity . Kamoi et al. [14] reported that there were no differences in the increasing rate of wall thickness of the bilateral ventricular walls and IVS between gestational trimesters but the rate was higher in the LV wall than in the RV wall over the entire course of gestation; the author suggested that the LV myocardium is dominant to that of

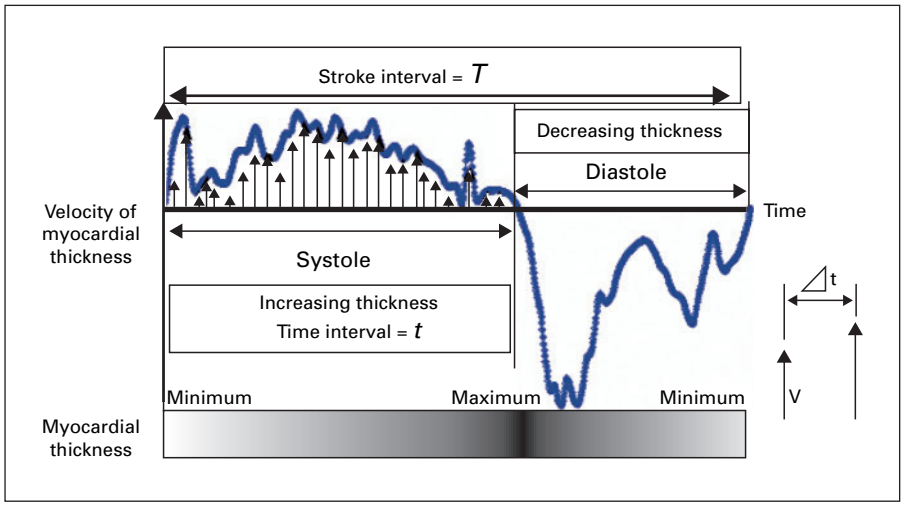

Fig. 1. Principle of average myocardial thickness velocity (v) and RITS. Δt: Time interval of measuring myocardial thickness velocity (222 μs). v: Velocity of myocardial thickness. Calculated average of V during systolic interval. RITS: (Increasing thickness interval:t)/(stroke interval:T).

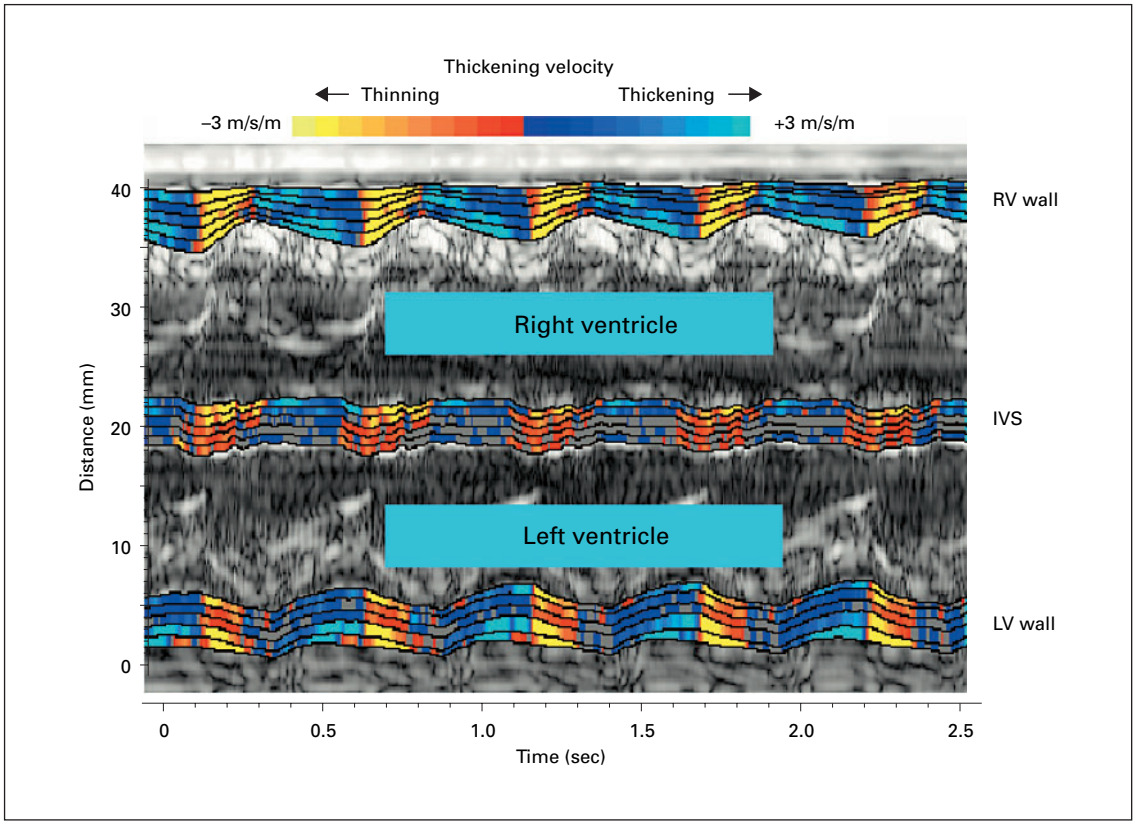

Fig. 2. Results of the phased tracking method. The ventricular wall is divided into layers 0.75 mm thick, and thickness changing rate is color coded: blue, increasing thickness; red and yellow, thinning; light blue and yellow, faster change. From top to bottom, these waves indicate the RV wall, IVS, and LV wall.

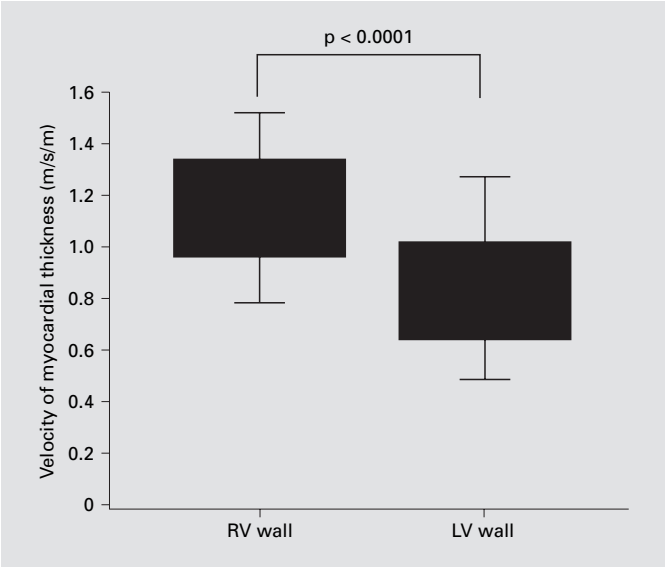

Fig. 3. Comparison of RV wall and LV wall myocardial thickness velocity. In these normal fetuses (n = 50), mean contraction rate was 1.18 ± 0.34 m/s/m (mean ± SD) in the RV wall and 0.86 ± 0.31 m/s/m in the LV wall, a significant difference ($p < 0.0001$).

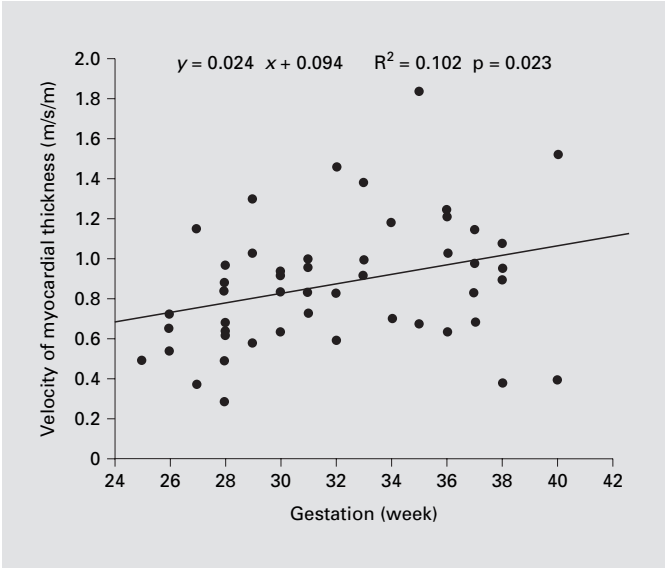

Fig. 5. Correlation between velocity of myocardial thickness in LV wall and gestational age (weeks).

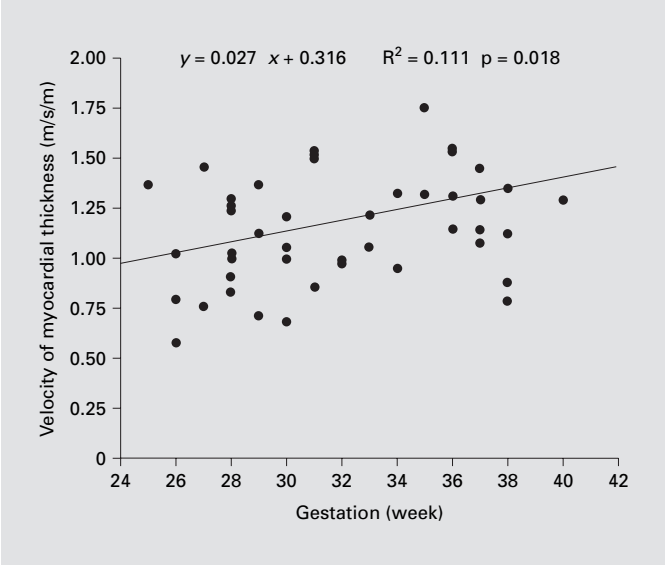

Fig. 4. Correlation between velocity of myocardial thickness in RV wall and gestational age (weeks).

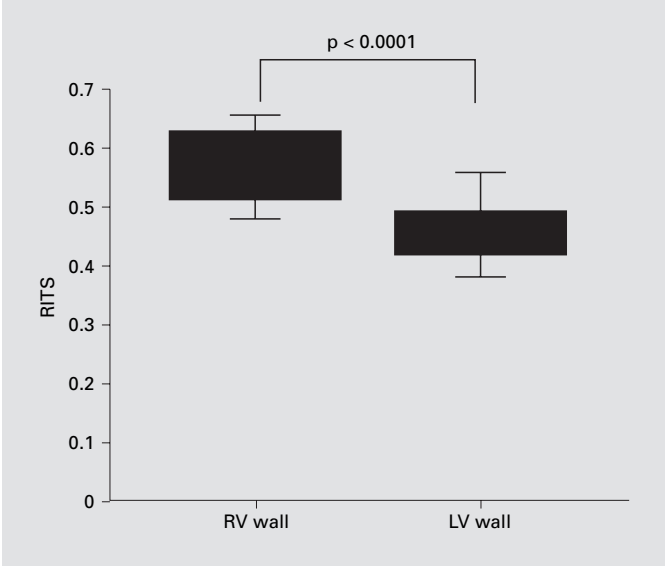

Fig. 6. RITS in the RV and LV walls. Mean RITS was significantly higher in the RV wall (0.57 ± 0.064) than in the LV wall (0.46 ± 0.075) (n = 50) ($p < 0.0001$).

the RV during fetal development as a pre-stage of LV functional dominance after birth.

Although these studies used almost the same method, the results differed. This may be because measurement of changes in the thickness of fetal cardiac muscle (considered to be about 1–2 mm wide) was difficult at the distance resolution of conventional ultrasound diagnostic instruments, taking into consideration the vertical dis-

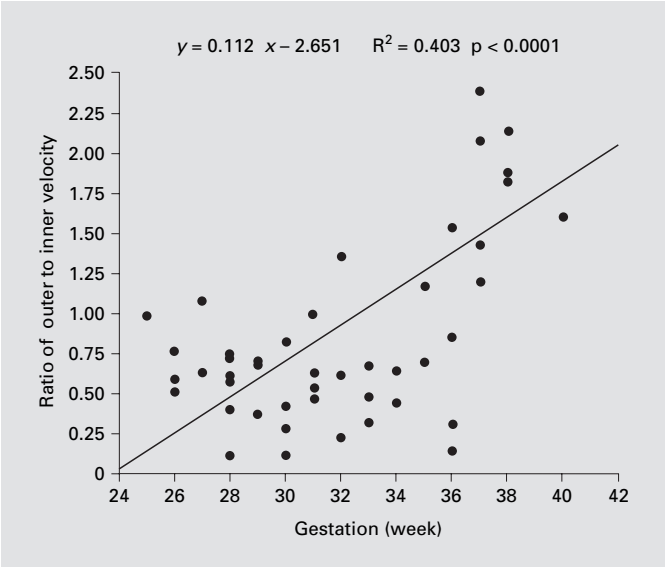

Fig. 7. Ratio of thickness changing rate of the outer layer to the inner layer of the RV wall (n = 50) with gestational age (weeks).

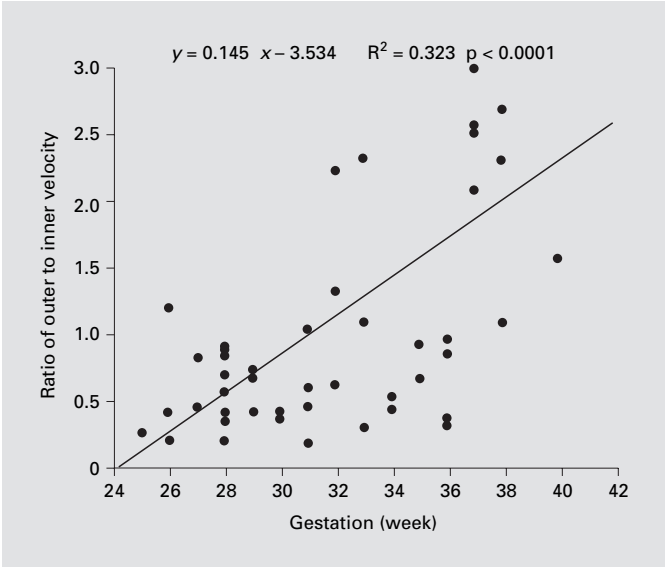

Fig. 8. Ratio of thickness-changing rate of the outer layer to the inner layer of the LV wall (n = 50) with gestational age (weeks).

tance resolution of about 1 mm in currently used ultrasound diagnostic instruments. The thickness-changing rate of the IVS wall was significantly lower than that of the RV and LV walls, suggesting that the septum was not positively involved in ejection from the ventricles. A study by Vosters [15], in which M-mode echocardiography was used, also reported that the wall thickening rate of the IVS during systole was significantly low.

Because ejection time has been reported as reduced in adult patients with heart failure [16], measurement of the systolic time interval by electrocardiography and Doppler echocardiography has been widely used for clinical evaluation of cardiac function. To evaluate cardiac function in fetuses, a method using the difference in blood flow time around the valve, called the Tei index, and the ratio was reported [17]. The Tei index in fetuses with intrauterine growth restriction and fetuses with gestation complicated by diabetes differs from those seen in normal fetuses, suggesting that this index can be used for screening of cardiac function abnormality in fetuses with high sensitivity [17]. It has also been reported that there is a significant difference in the Tei index between hydropic and normal fetuses, with the index correlated with the severity of heart failure [18]. However, because the Tei index returns to normal in severe heart failure, and simultaneous measurement of two or more blood flows in a single cardiac cycle is impossible when measuring the ratio of blood flow time around the valve, there are problems to be solved such as errors caused by the effect of changes in heart beat. Furthermore, because this method is an indirect evaluation of cardiac function using the blood flow interval, effects of pre- and post-load may not be avoided, suggesting that it is unclear whether evaluation of cardiac function with this method is accurate.

Because myocardial movement can be directly observed with high accuracy by the phased tracking method, myocardial contraction time can be accurately measured. Further, because simultaneous measurement of the RV and LV walls is possible with this method, changes in heartbeat may not affect measurement. Finally, because myocardial contraction time and thickness changing rate can be simultaneously measured, a more accurate and direct evaluation of cardiac function is possible.

In this study, we measured changes in myocardial thickness in normal fetuses by the phased tracking method, and we found more rapid and longer movements in the RV wall than in the LV wall. The RV is generally dominant in fetuses due to the direction of blood flow and output from the heart [19]. The results of our study indicate that fetal myocardial activity itself was also dominant in the RV.

In our preliminary experiment, we examined thickness changing rate of myocardium in sheep fetuses with acidemia caused by umbilical cord compression with use of the phased tracking method; the thickness changing rate and RITS in the RV were markedly reduced by ag-

gravation of blood gas conditions compared with those in the LV. Kamitomo et al. [20] reported that the output from the LV was not significantly lower in hypoxic sheep fetuses than in the control group, but RV output was significantly lower in the former group than in the latter. If reduction of contraction rate of the RV myocardium occurs prior to reduction of output, measurement of the myocardial thickening rate by the phased tracking method may be used for earlier diagnosis of hypoxia-induced functional RV insufficiency in fetuses. In fetuses with intrauterine growth restriction and in fetuses whose gestations are complicated by maternal diabetes, the relationship of thickness changing rate between the RV and LV were reversed (data not shown), suggesting that the normal dominance of the RV had almost failed.

With regard to evaluation of changes in the thickness of the local ventricular wall, there was a study in which the ventricular wall of an adult dog was divided into two or three layers using thread passing through the myocardium as the marker, and the wall thickening rate of the layers was measured using an ultrasonic diagnostic apparatus. The wall thickening rate of the inner layer was reported to be higher than that of the outer layer in adults [11, 21, 22]. In the present study, measurement of the thickness changing rate of local myocardium in fetuses by the phased tracking method indicated that the rate of the outer layer was higher than that of the inner layer during late gestation. Fisher et al. [23] examined the distribution of blood flow in the myocardium of sheep fetuses, newborn lambs, and adult sheep and reported that the distribution of local blood flow outside the myocardium was significantly higher in sheep fetuses in late gestation than in the newborn lambs and adult sheep [4]. Because the distribution of blood flow in local myocardium is considered to be related to oxygen consumption, contraction of the outer layer of myocardium may be more active in late gestation than after birth. Hay and Low [24] reported that the construction of denser and more regular filaments and their arrangement in a more fixed direction were observed in myofibrils in non-dividing myocardial cells as gestation proceeded and that these changes were more marked in the outer myocardial layer. The higher thickness changing rate of the outer layer of myocardium in late gestation may be caused by the high distribution of blood flow in the outer layer as well as physiological changes such as more rapid maturity of the myocardial microstructure.

The evaluation of local myocardium by the phased tracking method has just started, and its importance will be clarified by studying measurement of the ventricular walls in fetuses with disorders and in neonates.

In this study, we measured changes in the thickness of fetal myocardium by the phased tracking method and confirmed that the thickening changes were more rapid and longer in the RV wall than in the LV wall. Secondly, the thickness changing rate of the outer layer of myocardium increased with progression of gestation. This study suggests that measurement of changes in the thickness of fetal myocardium might be a good diagnostic tool for the evaluation of fetal cardiac function.

References

1 Beeby AR, Dunlop W, Heads A, Hunter S: Reproducibility of ultrasonic measurement of fetal cardiac hemodynamics. Br J Obstet Gynecol 1991;98:807–814.

2 Groenenberg IA, Hop WC, Wladimiroff JW: Doppler flow velocity waveforms in the fetal cardiac outflow tract: reproducibility of waveform recording and analysis. Ultrasound Med Biol 1991;17:583–587.

3 Simpson JM, Cook A: Repeatability of echocardiographic measurement in the human fetus. Ultrasound Obstet Gynecol 2002;20:332–339.

4 Harada K, Tsuda A, Orino T, Tanaka T, Takada G: Tissue Doppler imaging in the normal fetus. Int J Cardiol 1999;3:227–234.

5 Paradini D, Lamberti A, Teodoro A, Arienzo M, Tartagrione A, Martinelli P: Tissue Doppler imaging of the fetal heart. Ultrasound Obstet Gynecol 2000;16:530–535.

6 Tutschek B, Zimmermann T, Buck T, Bender HG: Fetal tissue Doppler echocardiography: detection rates of cardiac structures and quantitative assessment of the fetal heart. Ultrasound Obstet Gynecol 2003;21:26–32.

7 Twing P: Myocardial motion imaging: a new application of power color flow and frequency-based color flow Doppler in fetal echocardiography. Ultrasound Obstet Gynecol 1999;13:255–259.

8 Kanai H, Sato M, Koiwa Y, Chubachi N: Transcutaneous measurement and spectrum analysis of heart wall vibrations. IEEE Trans UFFC 1996;43:791–810.

9 Kanai H, Hasegawa H, Chubachi N, Koiwa Y, Tanaka M: Noninvasive evaluation of local myocardial thickening and its color coded imaging. IEEE Trans UFFC 1997;44:752–768.

10 Koiwa A, Kanai H, Hasegawa H, Saitoh Y, Shirato K: Left ventricular transmural systolic function by high sensitivity velocity measurement 'phased tracking method' across the septum in doxorubicin cardiomyopathy. Ultrasound Med Biol 2002;28:1395–1403.

11 Sabbah HN, Marzilli M, Stein PD: The relative role of subendocardium and subepicardium in left ventricular mechanics. Am J Physiol 1981;240:920–926.

12 Winsberg F: Echocardiography of the fetal and newborn heart. Inves Radiol 1972;7:152.

13 Sutton MG, Gewitz MH, Shah B, Cohen A, Reichek N, Gabbe S, Huff DS: Quantitative assessment of growth function of the cardiac chambers in normal human fetuses. Circulation 1984;69:685.

14 Kamoi S: Fetal development and fetal heart function analyzed by M-mode echocardiography. Acta Obst Gynaec Jpn 1989 41:799–805.

15 Vosters R, Wladimiroff JW, Versprille A: M-mode ultrasound recording of perinatal geometry and dynamics of the cardiac interventricular septum. Eur J Obstet Gynecol Reprod Biol 1984;16:299.
16 Arnold M, Willard H, Clyde S: Systolic time intervals in heart failure in man. Circulation 1968;37:149.
17 Tutumi T, Isii M, Eto G, Hota M: Serial evaluation for myocardial performance in fetuses and neonates using a new Doppler index. Pediatrics Int 1999;42:722–727.
18 Falkensammer CB, Paul J, Huhta JC: Fetal congestive heart failure: correlation of Tei-index and Cardiovascular-score. J Perinat Med 2001;29:390–398.
19 Kamitomo M, Longo LD, Gilbert RD: Cardiac function in fetal sheep during two weeks of hypoxemia. Am J Physiol 1994;266:1778–1785.
20 Kamitomo M, Longo LD, Gilbert RD: Right and left ventricular function in fetal sheep exposed to long term high-altitude hypoxemia. Am J Physiol 1992;262:399–405.
21 Gallagher KM, Osakada G, Matsuzaki M, Miller M, Kemper WS, Ross J Jr: Nonuniformity of inner and outer systolic wall thickening in conscious dog. Am J Physiol 1985;249: H241–H248.
22 Myers JH, Stirling MC, Choy M, Buda AJ, Gallagher KP: Direct measurement of inner and outer wall thickening dynamics with epicardial echocardiography. Circulation 1986;74:164–172.
23 Fisher DJ, Heymann MA, Rudolph AM: Regional myocardial blood flow and oxygen delivery in fetal, newborn, and adult sheep. Am J Physiol 1982;243:H729–H731.
24 Hay DA, Low FN: The fine structure of progressive stages of myocardial mitosis in chick embryos. Am J Anat 1972;134:175–201.